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Article (Accepted Version)

Coleman, Jonathan R I, Lester, Kathryn J, Roberts, Susanna, Keers, Robert, Lee, Sang hyuck, De Jong, Simone, Gaspar, H  l  na, Teismann, Tobias, Wannem  ller, Andr  , Schneider, Silvia, J  hren, Peter, Margraf, J  rgen, Breen, Gerome and Eley, Thalia C (2016) Separate and combined effects of genetic variants and pre-treatment whole blood gene expression on response to exposure-based cognitive behavioural therapy for anxiety disorders. *World Journal of Biological Psychiatry*, 18 (3). pp. 215-226. ISSN 1562-2975

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To cite this article: Jonathan R.I. Coleman, Kathryn J. Lester, Susanna Roberts, Robert Keers, Sang hyuck Lee, Simone De Jong, Helena Gaspar, Tobias Teismann, André Wannemüller, Silvia Schneider, Peter Jöhren, Jürgen Margraf, Gerome Breen & Thalia C. Eley (2016): Separate and combined effects of genetic variants and pre-treatment whole blood gene expression on response to exposure-based cognitive behavioural therapy for anxiety disorders., The World Journal of Biological Psychiatry, DOI: [10.1080/15622975.2016.1208841](https://doi.org/10.1080/15622975.2016.1208841)

To link to this article: <http://dx.doi.org/10.1080/15622975.2016.1208841>

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 Accepted author version posted online: 04 Jul 2016.
Published online: 04 Jul 2016.

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Word Count: 4977/5000 (Main); 200/200 (Abstract)

Figure Count: 1

Table Count: 6

Title: Separate and combined effects of genetic variants and pre-treatment whole blood gene expression on response to exposure-based cognitive behavioural therapy for anxiety disorders.

Short Title: Effects of baseline gene expression on CBT outcome

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Conflict of interest:

Dr Breen is a consultant in pre-clinical genetics for Eli Lilly. All other authors declare no financial interests.

Keywords: Genetics, Psychotherapy, Anxiety disorders, Gene expression, Exposure therapy

Abstract

Objectives

Exposure-based cognitive behavioural therapy (eCBT) is an effective treatment for anxiety disorders. Response varies between individuals. Gene expression integrates genetic and environmental influences. We analysed the effect of gene expression and genetic markers separately and together on treatment response.

Methods

Adult participants ($n \leq 181$) diagnosed with panic disorder or a specific phobia underwent eCBT as part of standard care. Percentage decrease in the Clinical Global Impression severity rating was assessed across treatment, and between baseline and a six month follow-up.

Associations with treatment response were assessed using expression data from 3,233 probes, and expression profiles clustered in a data-driven and literature-driven manner.

3,343,497 genetic variants were used to predict treatment response alone and combined in polygenic risk scores.

Genotype and expression data were combined in expression quantitative trait loci (eQTL) analyses.

Results

Expression levels were not associated with either treatment phenotype in any analysis.

1,492 eQTLs were identified with $q < 0.05$, but interactions between genetic variants and treatment response did not affect expression levels significantly.

Genetic variants did not significantly predict treatment response alone or in polygenic risk scores.

Conclusions

We assessed gene expression alone and alongside genetic variants. No associations with treatment outcome were identified. Future studies require larger sample sizes to discover associations.

JUST ACCEPTED

Introduction

Anxiety disorders are the most common group of mental illnesses, with lifetime prevalence estimates ranging between 10-30% (Kessler *et al* 2007; Michael *et al* 2007). They are an economic burden on society and the sixth largest cause of disability globally (Baxter *et al* 2014; Fineberg *et al* 2013). Suffering from an anxiety disorder is distressing, with affected individuals reporting adverse effects on quality of life comparable to sufferers of major depressive disorder, and in excess of the population norm (Mendlowicz and Stein 2000).

Treatment of anxiety disorders uses a variety of pharmacological and psychological modalities (National Collaborating Centre for Mental 2011). Exposure-based cognitive behavioural therapy (eCBT) is a common treatment, and shows large effect sizes across the anxiety disorders, comparable to or better than those obtained by anxiolytic medication (Barlow *et al* 2013; Cuijpers *et al* 2013; Margraf and Zlomuzica 2015; Norton and Price 2007; Stewart and Chambless 2009). During eCBT, participants confront the object of their anxiety (whether literally, referred to as *in vivo*, or through imagination or virtual reality, referred to as *in sensu*), within a carefully managed and supportive environment. They identify the cognitive and behavioural processes underlying their anxious response, and develop strategies to mitigate against these negative schema and to cope with their anxiety (Otto *et al* 2004). Rates of response (in terms of a reduction in symptom severity) and of remission (no longer meeting diagnostic criteria) vary between specific disorders and studies, but are invariably less than 100% (Ballenger 1999; Hofmann *et al* 2012; Loerinc *et al* 2015; Olatunji *et al* 2010).

Numerous influences have been proposed to lead to poorer treatment outcome, including high initial severity, Axis I and Axis II comorbidity, illness duration, low expectancy of

treatment success, poor treatment compliance and therapeutic alliance, and general interpersonal difficulties (Newman *et al* 2013). However, studies disagree on the importance and validity of such predictors (Olatunji *et al* 2013; Schneider *et al* 2015; Taylor *et al* 2012). The success of any specific treatment for a given participant is difficult to predict. This is relevant given the high costs (both economic and emotional) of pursuing unsuccessful treatment (Otto *et al* 2000). It is of clear interest to develop reliable predictors of treatment response.

Genetic variants represent a potential source of predictors. The study of such variants (termed therapygenetics) has largely been confined to candidate gene studies (Eley *et al* 2012; Lester and Eley 2013). However, these findings have proven difficult to replicate, and the direction of effect found has been inconsistent between studies (Lester *et al* 2016). Recently, we published a GWAS of therapy response in a cohort of children with anxiety disorders (Coleman *et al* 2016). Although underpowered to identify the small-effect variants typical of behavioural phenotypes, sufficient power was available to test some effect sizes reported in the therapygenetic literature. No variants were found at conventional genome-wide significance, and candidate variants were not replicated. Therefore, the effects of individual genetic variants on response to CBT are likely to be small, and that the predictive effects of such variants are likely to be negligible when used alone.

Studying the differential expression of gene transcripts may be more useful for predicting treatment response. Multiple factors affect gene expression, potentially including genetic variants and environmental influences. Gene expression represents a biologically relevant means of combining genetic and environmental variation to predict response to CBT for anxiety disorders. Two studies have found an association between increased expression of FKBP5 and response to CBT for PTSD (Levy-Gigi *et al* 2013; Yehuda *et al* 2013). A recent analysis of change across

treatment including a subset of the cohort presented within this paper showed no association between treatment response and individual gene expression, nor when expression was clustered according to similarities in expression in the data (Roberts *et al* Under Review).

This investigation combines genetic and gene-expression approaches to predict response to eCBT. It assesses the interaction of differential gene expression at baseline (both of individual transcripts, and using data-driven and literature driven clustering methods) and genetic variation to assess the outcome of eCBT for panic disorder and specific phobias.

Method

Participants and therapeutic procedure

244 participants diagnosed with panic disorder or a specific phobia completed one of four exposure-based CBT treatment programs at the Mental Health Research and Treatment Center, Ruhr-Universität Bochum, Germany as part of standard care. In all programs, diagnoses were made according to DSM-IV criteria using the Diagnostisches Interview bei psychischen Störungen (DIPS) and Mini-DIPS, structured interviews with well-established reliability, validity and patient acceptance (Bruchmuller *et al* 2011; In-Albon *et al* 2008; Margraf 1994; Schneider and Margraf 2011; Suppiger *et al* 2009; Suppiger *et al* 2008). All treatment programs featured core elements of exposure therapy, including psychoeducation, applied relaxation and exposure (*in vivo* or *in sensu*). Specifics of each treatment program are described below. All treatments were regularly supervised by experienced senior clinicians using audio-visual recordings in order to ensure treatment protocol integrity.

Individuals diagnosed with a specific phobia of receiving dental treatment, not secondary to a separate diagnosis (such as PTSD or injection phobia), were treated in a dental anxiety-specific

program (DA; (Wannemuller 2015)). Treatment was given in five weekly sessions comprising an initial diagnostic and psychoeducation session, a session developing relaxation techniques, and three *in sensu* exposure sessions related to dental treatment. Participants were not excluded on the basis of concurrent treatment with anxiolytic medication.

Participants with specific phobia (SP) not primarily associated with dental fear were treated in a longer-term program covering up to thirty sessions, split into five initial sessions of diagnosis and psychoeducation, and twenty-five sessions of *in vivo* exposure (relevant to their specific phobia) with elements of cognitive restructuring. Participants were excluded from the study if they were using anxiolytic medication.

Participants with a primary diagnosis of panic disorder with agoraphobia, or agoraphobia alone, were randomized either to exposure-based CBT (PD-CBT; akin to the specific phobia group) or to an exposure-alone condition without any element of cognitive restructuring (PD-EXP; Clinical Trials: NCT01680327). Participants in both conditions were excluded if they were using anxiolytic medication. Bodily sensation was used as the specific exposure stimulus for participants suffering from panic disorder. However, as there were no patients with panic disorder without agoraphobia in the trial, interoceptive exposure was always combined with *in vivo* exposure.

Prior to receiving exposure, immediately following completion of the treatment program, and at a follow-up assessment approximately six months after treatment completion, all participants completed a range of questionnaire measures. In addition, peripheral blood was drawn for DNA and RNA extraction.

Phenotype definition

Treatment response was defined as percentage improvement in the clinician-rated severity scale of the Clinical Global Impression rating (CGI-S), and was examined pre-treatment to post-treatment, and pre-treatment to follow-up. The CGI-S ranges from 1-7, with a score of 1 representing no symptoms of concern and a score of 7 representing extremely severe illness requiring hospitalization (Guy 1976). The scale was chosen as it was used in all treatment groups, and was expected to capture severity in a disorder-independent fashion.

The CGI-S was rescaled to range from 0-6 to allow outcome to be defined as percentage decrease in severity across time (as this has previously been used successfully in pharmacogenetic GWAS) with 100% indicating full remission (Uher *et al* 2010). For both phenotypes, correlations were calculated between percentage improvement and a variety of covariates: age, gender, severity at baseline, presence of comorbid mental disorders, number of treatment sessions attended, treatment period (days between pre-treatment and post-treatment assessment), follow-up period (days between post-treatment assessment and follow-up assessment), use of psychoactive medication at pre-treatment, use of any other medication pre-treatment, body mass index and whether the participant smoked. Although the use of concurrent anxiolytic medication was an exclusion criterion for the SP, PD-CBT and PD-EXP groups, some participants were using other medications which may have a psychotropic effect, so this covariate was not restricted to the DA group (Table 1).

Of these covariates, severity at baseline, presence of comorbid mental disorders, use of psychoactive medication and follow-up period were correlated with at least one phenotype in the whole cohort (Table 2). In secondary examinations within each treatment group, treatment period

was associated with at least one phenotype in both the PD-CBT ($p=0.014$) and SP groups ($p=0.012$). Body mass index (BMI) was weakly associated ($p=0.0424$) with response at post-treatment in the PD-CBT group; however, as this effect was not seen in any other group nor in the whole cohort, BMI was not included as a covariate.

The phenotypes for analysis were defined as the residuals from two linear mixed regressions investigating change in severity between pre-treatment and post-treatment, and pre-treatment and follow-up. Percentage decrease in severity was regressed on fixed effects of baseline severity, presence of comorbid mental disorders, use of psychoactive medication, treatment period (and follow-up period in the analysis pre-treatment to follow-up), and a higher-order random effect of treatment group (to account for differences between treatment groups).

Genotyping

DNA was extracted from peripheral blood drawn pre-treatment using Qiagen FlexiGene DNA kits, following the protocol provided by the manufacturer (Qiagen Ltd). DNA concentration was quantified using spectrophotometry (Nanodrop 1000, Nanodrop, Wilmington, DE), and samples diluted to 40 μ l at a concentration of 75ng/ μ l for genotyping. Genotyping was performed using the Illumina PsychChip microarray (Illumina, San Diego, CA), a modified version of the Illumina HumanCoreExome microarray with additional content of interest in psychiatric genomics. All laboratory procedures were performed at the Institute of Psychiatry, Psychology and Neuroscience, KCL.

Genotype quality control

Quality control was performed following a previously published protocol (Coleman *et al* 2015). In brief, genotype data was called using Illumina GenomeStudio software, with manual recalling where appropriate. Rare variants were recalled using ZCall (Goldstein *et al* 2012). Variants were removed from the analysis if they were rare (minor allele frequency < 0.05), present in < 99% of individuals, or deviated substantially from Hardy-Weinberg equilibrium (Hardy-Weinberg test $p < 1 \times 10^{-5}$). Individuals were excluded if they had genotype calls for < 99% of variants, where reported gender differed from that indicated by the genotypes, or if genome-wide estimates of heterozygosity > 3 standard deviations from the sample mean. Additional exclusions were made if the individual showed cryptic relatedness to other individuals in the study (IBD > 0.1875) or had an average proportion of variants shared IBD with the cohort as a whole > 6 standard deviations above the cohort mean.

Following quality control, variants were imputed to the Phase 3 release from the 1000 Genomes Project, using IMPUTE2 with concurrent phasing (1000GenomesConsortium 2012; Howie *et al* 2012). X chromosome variants were imputed using the March 2012 Phase 1 release (1000GenomesConsortium 2012; Howie *et al* 2012). Imputed variants were imported into PLINK2 for analysis, and filtered to remove uncertain variants (posterior-probability < 0.8) and poorly-imputed variants (info < 0.8) (Chang *et al* 2015). Following hard-calling, variants present in < 98% of the cohort were dropped from analysis.

Gene expression

Whole blood samples were drawn at pre-treatment using PAXgene blood RNA tubes. Blood RNA was isolated and purified using the PAXgene Blood miRNA Kit according to the

manufacturer's protocol using the Qiagen Qiacube (Qiagen). RNA quality was measured using spectrophotometry (Nanodrop 1000, Nanodrop, Wilmington, DE) and integrity using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). Genome-wide expression levels were measured from 750ng total RNA using the Illumina HumanHT-12v4 Expression Beadchip (Illumina, San Diego, CA).

Gene expression quality control

Raw expression data were processed following internal pipelines (available at https://github.com/snewhouse/BRC_MH_Bioinformatics). Samples with detection rates dissimilar from the rest of the cohort were identified and removed in GenomeStudio (Illumina, San Diego, CA). Raw data was imported into R for quality control primarily using the lumi package (Du *et al* 2008; Team 2012). Expression data was background corrected using Module-based Background Correction for Beadarray (Ding *et al* 2008). Probes with an expression level >2 standard deviations above the background mean were defined as detected. *XIST* gene expression (specific to females) and Y chromosome gene expression (specific to males) was compared to reported gender and gender inferred from genotyping, and discordant samples removed. Expression data was log2 transformed and normalized using robust splines normalization from the lumi package (Du *et al* 2008; Schmid *et al* 2010). Sample co-expression relationships were assessed, and samples with connectivity <2 standard deviations from the cohort mean were excluded (Oldham *et al* 2012). Associations between covariates and the first principal component of the expression data were assessed using stepwise linear regression bootstrapped 100 times, with randomized order of covariates in the regression. Covariates included batch variables (expression microarray, sample position on microarray, date of RNA extraction, date of expression measurement, machine used in RNA isolation, RNA integrity

(RIN) value, RNA yield, amplified concentration of RNA, whether the sample required additional treatment to remove DNA and whether the blood sample was the first or second drawn) and demographic covariates (body mass index and smoking). The effect of associated covariates was regressed out of the expression data, using sva's ComBat package in the case of categorical variables (extraction date) and linear regression (with RcppArmadillo) in the case of continuous variables (RIN value, RNA yield, amplified concentration of RNA) (Eddelbuettel and Sanderson 2014; Johnson *et al* 2007). Probes detected in <80% of the sample were removed. As expression data was generated from whole blood without assessment of cellular composition, deconvolution methods implemented in CellMix were used to assess the origin of RNA transcripts before and after differentially expressed probes were selected (Gaujoux and Seoighe 2013). Correlations between the estimated final proportions of leukocytes (neutrophils, lymphocytes and monocytes) and the two CBT response phenotypes were calculated. Additional exclusion of probes was performed to allow combined analysis with genotyped variants. Specifically, probes were excluded if they were not annotated in the ENSEMBL hg19 build, if they contained any genetic variant genotyped in the cohort, or if they did not map to a unique site on the genome. Probes were identified using nucleotide universal identifiers (nuIDs), which are unique to the DNA sequence of the probe (Du *et al* 2007).

Statistical analysis

Following quality control, the association of genome-wide genotyping data with both response phenotypes was assessed in genome-wide association studies (GWAS). The participants in the study were of Central or Eastern European ancestry. Genomic estimation of ancestry was established using principal components analysis performed in EIGENSOFT (Price *et al* 2006). No principal component was correlated with either of the phenotypes at a level greater than

chance. To account for finer-scale population stratification, analyses were run using a linear mixed model incorporating a random effect of gross genetic similarity between individuals (the *mlma-loco* option in GCTA (Yang *et al* 2011)). Results were clumped in PLINK2, pruning all variants in linkage disequilibrium ($r^2 > 0.25$, +/- 250 kb) of a variant with a lower *p*-value.

Genotype information was used as a target dataset in polygenic risk scoring. Specifically, the results of a previous GWAS of CBT response in children were used to predict both phenotypes in the whole cohort using PRSice, which performs high-resolution polygenic risk scoring to identify the most predictive risk score (Coleman *et al* 2016; Euesden *et al* 2015). Further GWAS were performed on the cohort minus individuals treated for dental anxiety, and the results from these subset GWAS were used to predict response to treatment in the dental anxiety subgroup.

Probe-level expression data was imported into R, and analysed using Weighted Gene Correlation Network Analysis (WGCNA (Langfelder and Horvath 2008)). Data-driven clustering of co-expressed probes was performed using an automatically-constructed signed network from the *blockwiseModules* function in WGCNA (details on this procedure are provided in the Supplementary Methods (Langfelder and Horvath 2008)). Correlations between individual probes and both response phenotypes, and between WGCNA module eigengenes and response phenotypes, were calculated. Local false discovery rates were calculated to account for multiple testing using the *qvalue* package in R (Dabney *et al* 2004).

Probe-level correlations were mapped to HUGO gene names, ranked according to significance and used in Gene Ontology enrichment analysis in GOrilla (Eden *et al* 2009). Where multiple probes mapped to the same gene, the highest-ranked was retained. Details of the enrichment analysis performed by GOrilla are provided in the Supplementary Methods. Significance was set

as the Bonferroni correction for the 8746 GO terms tested ($p=5.72 \times 10^{-6}$), with results reported below $p=5 \times 10^{-4}$. Results were pruned for redundancy in REVIGO, with results with >50% dispensability dropped (Supek *et al* 2011).

Probe-level expression data was combined using a machine-learning approach in WEKA, to assess the viability of prediction from expression probe data alone (Hall *et al* 2009). Classical machine learning algorithms were used to predict outcome using the full dataset (3,233 expression probes, 166 participants for baseline to post-treatment analysis, 110 participants for baseline to follow-up analysis). Five approaches were used: mean prediction with ZeroR; inverse distance weighting with a nearest neighbours algorithm (kNN), with and without subset evaluation; linear kernel-based regression with Regression SVM (SMOReg) and a 500-tree Random Forest algorithm. Multiple algorithms were chosen as they optimise different aspects of the learning process. All analyses were performed using 10-fold-cross validation (splitting the cohort into 80% training and 20% test subsamples), repeated 5 times.

Probe-level expression data and genotype data were imported into R for eQTL analyses using the MatrixEQTL package (Shabalin 2012). All transcripts captured by the assessed probes were mapped to the hg19 build of the human genome. Analyses were performed using a two-stage design. *Cis*-eQTLs were calculated independent of the phenotype, using the *modelLINEAR* option and genotypes from a window ± 100 kb of the transcript. Linkage-independent results were obtained by clumping using PLINK2 (250kb window, $r^2 < 0.25$), and by performing conditional eQTL analyses (Chang *et al* 2015). Both techniques identified the same sentinel SNPs (data not shown). Clumped results were retained for the second, phenotype-dependent, stage. SNP-by-treatment response interactions predicting expression change were assessed for both phenotypes,

using the *modelLINEAR_CROSS* option in MatrixEQTL, to investigate whether the effect of eQTLs in the data differed in relation to treatment response.

Power analyses for the expression analyses were performed using the pwr package in R.

Ethics

Ethics approval for this study was received from the Ethics Committee at the Faculty of Psychology, Ruhr-Universität Bochum, from the London-Bentham NRES Committee and from the King's College London Psychiatry, Nursing and Midwifery Research Ethics Sub-Committee. All participants provided informed consent. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Results

Phenotype data was available on 187 participants (185 at post-treatment; 122 at follow-up). Following quality control, genotype data was available on 3,343,497 variants (267,037 genotyped) for 181 participants for the post-treatment analysis (122 were available for the analysis at follow-up). Data from 3,233 expression probes were available on 166 (110) participants. Both data types were available on 162 (110) participants.

Demographics and clinical covariates

Demographic data on the cohort are displayed in Table 1. Individuals in the SP group had lower baseline severity than all other groups. Groups also differed by mental disorder comorbidity, with individuals in the DA group exhibiting more comorbidity than other groups, and significantly more than the SP group (details of mental comorbidities are provided in Supplementary Table 1). As expected, there was a higher rate of psychoactive medication use in

the DA group compared to all others. Treatment duration also differed significantly across the groups, with shorter treatment in the DA group than in all others. Follow-up duration was significantly longer in the DA group. All covariates showing inter-group differences were included as covariates when defining the treatment response phenotypes (as was a random effect of treatment group; Table 2).

[Approximate position of Tables 1 and 2]

Changes in CGI from pre-treatment to post treatment and to follow-up are described in Table 3. All treatments were generally effective, with most participants improving on the CGI-S between pre-treatment and post-treatment, and between pre-treatment and follow-up. However, there was considerable variance in the percentage change shown between individuals. Demographic differences between response groups following treatment are described for the whole cohort in Supplementary Table 2. Significantly lower baseline severity and higher comorbidity was observed in those deteriorating compared to those improving. No other significant differences were observed.

[Approximate position of Table 3]

GWAS and polygenic risk score analysis

Results from both GWAS are shown in Supplementary Table 3, and Supplementary Figures 1 and 2. No variants passed the threshold for genome-wide significance ($p=5\times10^{-8}$), but three independent loci in the analysis to post-treatment and four loci in the analysis to follow-up reached a suggestive level of significance ($p<5\times10^{-6}$). Quantile-quantile plots indicated no substantial genomic inflation in either analysis.

Polygenic risk score analysis from an independent GWAS of response to CBT in children failed to predict response in the whole cohort with $p < 0.001$ (Supplementary Table 4a, threshold adjusted for multiple testing (Euesden *et al* 2015)). Prediction between the DA treatment group and all other treatment groups explained more variance in outcome than the analysis using the independent GWAS, but predictors were not significant (Supplementary Table 4b). Further discussion of the GWAS and PRSice analyses are included in the Supplementary Material.

Individual expression probes

No probes were significantly associated with either phenotype after correcting for multiple testing (all $q > 0.05$; Table 4). The probes with the lowest q -values in this analysis showed no overlap with those reported in a parallel analysis of this cohort, examining change in expression over the course of treatment (Roberts *et al* Under Review).

Power analyses indicated the analyses have 80% power to detect associations capturing at least 14.8% (post-treatment), and 19.6% (follow-up) of variance respectively, where $\alpha = 1.55 \times 10^{-5}$ (Bonferroni-correction for 3233 tests).

[Approximate position of Table 4]

Data-driven network-based analyses

Clustering by co-expression patterns yielded eight network modules ranging from 750 to 63 probes and a further "grey" module of 459 probes that did not fall into any cluster. Although different clusters showed associations with a variety of sample characteristics, no cluster was associated with either treatment response phenotype (all $p > 0.05$; Figure 1).

[Approximate position of Figure 1]

Literature-driven gene ontology analysis

HUGO gene names were assigned to 2,652 probes associated with at least one GO term (process, function or component). No significant pathways were found after correction for multiple testing (all $p > 5.72 \times 10^{-6}$). Following removal of redundant GO terms, five processes and one function were associated with $p < 5 \times 10^{-4}$ in the analysis from baseline to post-treatment. From baseline to follow-up, eight processes and two functions were associated with $p < 5 \times 10^{-4}$ (Supplementary Table 5).

Classical machine learning analyses

Classical machine learning methods did not outperform the null model in either analysis. The most effective model was random forest classification (root mean square error: 31.3 (post-treatment), 42.7 (follow-up)) but this did not outperform ZeroR, which predicts the mean (RMSE: 30.6 (post-treatment), 42.3 (follow-up)).

Expression quantitative trait loci

Expression quantitative trait locus (eQTL) analysis identified 42,868 *cis*-eQTLs with $q < 0.05$, independent of phenotype. Following the removal of variants in linkage disequilibrium with more strongly associated eQTLs, 1,492 variants were present with $q < 0.05$ (Table 5, Supplementary Table 5). Phenotype-dependent analyses of the interaction between these variants and treatment response predicting expression levels yielded no associations with $q < 0.05$ (Table 6). One interaction was identified with $q < 0.2$ (rs10498246 x treatment response baseline to follow-up, predicting *SP110* (probe nuID: fcV3S0U75If1e3op0U) expression, $B = -0.0041$, $p = 2.23 \times 10^{-5}$, $q = 0.103$).

[Approximate position of Tables 5 and 6]

Discussion

We performed genome-wide analysis of genetic variation and pre-treatment gene expression to assess independent and combined effects on response to CBT for anxiety disorders in a cohort of adult participants. This is the first analysis to integrate this data in studying psychological treatment response, and (together with a companion paper; (Roberts *et al* Under Review)) is an analysis of the largest psychological treatment cohort in which gene expression analyses have been performed. Despite this, no variants or expression profiles were associated (at a genome-wide level of significance) with treatment response across the treatment period or at a six-month follow-up.

The cohort is larger than previous studies of the effect of gene expression on response to CBT in anxiety disorder (Levy-Gigi *et al* 2013; Yehuda *et al* 2013). However, it is clear that this study is underpowered to detect all but the largest effects on response, and that robust prediction requires larger cohort sizes. Integrating data from two different approaches (that is, genotyping and gene expression) increases power, but requires two sets of quality control, resulting in fewer samples with full data available (Ritchie *et al* 2015). Obtaining a large sample size for a study such as this is non-trivial. Prospective recruitment results in a high rate of attrition as participants withdraw from treatment or are lost to follow-up. Furthermore, this attrition is likely to be related to poor treatment response.

The aim of this investigation was to study genetic and transcriptomic correlates of response to exposure-based therapy, which may act across diagnostic boundaries. We sought to increase power by recruiting from treatment studies for two disorders with differing treatment procedures.

Studying anxiety disorders as a heterogeneous group has been effective in genomics (Otowa *et al* 2016). However, combining groups increases heterogeneity, partially negating the increased power from the enlarged sample size. The disorders studied are conceptually distinct, and treatment is tailored to the needs of the participant, differing between and within diagnostic groups. Combining across disorder groups incurs disorder-specific differences, such as the lower baseline severity of the SP group and higher comorbidity in the DA group herein. These differences reflect the varying nature of the disorders and recruitment to treatment – for example, the high comorbidity of the DA group is likely to result from secondary consequences of avoiding dental treatment, such as a phobia of vomiting or social anxiety about visiting the dentist. Although we have sought to control for this heterogeneity statistically, it limits the conclusions of this investigation. Furthermore, many social and environmental influences on treatment response have been proposed, and the covariates controlled for within this analysis cannot correct for all possible confounds. Nevertheless, investigating biological correlates of therapy requires a pragmatic approach. Cohorts of individuals receiving psychological therapies, particularly those outside of clinical trials, are prone to heterogeneity and attrition. For any biological predictor to contribute valuably to therapeutic decision-making, it must be robust to these limitations.

Although no genome-wide gene expression studies have investigated response to CBT, single-gene studies have suggested a role for differential *FKBP5* expression in response to CBT for PTSD (Levy-Gigi *et al* 2013; Yehuda *et al* 2013). One probe in this study, Zdi45Se3VG7s869FKo, captures expression of FKBP5, but was not associated with either outcome (baseline to post-treatment: $p=0.0533$, $q=0.999$; baseline to follow-up: $p=0.607$,

$q=0.997$). However, the low power of the analysis (and differences between this cohort and those examined previously), limit strong conclusion..

Gene expression differs between different tissues and organs; expression observed in peripheral blood may not reflect that in the brain. Previous studies suggest moderate correlation between gene expression in different tissues, varying by individual genes (Sullivan *et al* 2006). The emergence of reference panels such as the GTEx Portal has made *in silico* assessment of blood-brain expression correlations at the individual gene level viable (Consortium 2015). As such, peripheral blood gene expression can provide relevant insights into gene expression in the brain, and this will improve as further brain expression samples are added to the reference. From a pragmatic standpoint, gene expression markers of treatment response will only be useful if they can be obtained from peripheral tissues – while the effect of gene expression in brain tissues is of biological interest, it cannot be of practical utility in this case.

Assessing the severity of anxiety disorders can be performed using different rating scales, with varying characteristics. No consensus regarding the best means of measuring response to CBT exists (Loerinc *et al* 2015). In this study, the CGI-S was used as a measure of clinical concern across treatment groups, allowing a single measure to be used to assess general functioning. However, this measure bears a number of limitations. It is a subjective measure of clinical judgement that may fail to capture the participant's anxiety as appropriately as a self-report measure. Treatment response is likely to involve multiple components, including reduction in fear and increase in functioning, that a single measure may not capture. One potential solution is to combine a number of scales using different assessors and assessing different aspects of treatment response. However, this increases the complexity of the analysis and the potential for spurious results. In addition, it would be difficult to interpret in a useful manner.

Anxiety disorders are widespread and disabling, and CBT is a first-line treatment for these conditions. CBT involves a considerable investment from the recipient, and a significant minority of those receiving it do not respond adequately. Stable pre-treatment predictors of outcome are required. To date, genetic variants and gene expression levels have not provided these predictors, individually or in combination. However, this does not argue against the continued study of the biology underlying CBT response. The pattern of findings to date is consistent with the highly polygenic model that has been proposed to influence behavioural traits (Chabris *et al* 2015). Although individual genetic variants seem extremely unlikely to be valuable predictors, prediction might be achieved through the combined effect of many genetic variants, at multiple levels of analysis.

Response to CBT is likely to be influenced by genes and by the environment, and continued research to define reliable environmental and clinical predictors of response is vital –genetics can only be clinically useful in the context of known environmental and clinical risk factors (Hudson *et al* 2015). Studies of genetic variation, gene expression and epigenetics should either adopt a hypothesis-neutral approach (exploring variation genome-wide), or be informed by robust associations in related traits (rather than assumed biological relevance). The effects of individual transcript differences are likely to be small. For these insights to be discovered, cohorts of thousands of individuals must be treated in as homogenous a manner as possible from recruitment to the analysis of the resulting data. This is not straightforward (especially given the heterogeneity inherent to CBT) but the example of the many international consortia driving advances in complex trait genetics demonstrates such investigations can yield valuable insights.

Acknowledgements

This paper represents independent research part supported by the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) at South London and Maudsley NHS Foundation Trust and King's College London, as well as the Alexander von Humboldt Foundation (Germany). JRIC's PhD is funded by the Institute of Psychiatry, Psychology and Neuroscience, and the Alexander von Humboldt Foundation. SDJ was funded by NARSAD Young Investigator Grant (YI 60373). We would like to thank Hamel Patel, Amos Folarin and Stephen Newhouse from the NIHR BRC Bioinformatics Core for their help and the use of their pipelines (<http://core.brc.iop.kcl.ac.uk/>). We acknowledge the use of the multi-node computing cluster maintained by the NIHR BRC Bioinformatics Core. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Summary statistics from all analyses are available on request from the authors.

Conflict of interest

Breen is a consultant in pre-clinical genetics for Eli Lilly. All other authors declare no financial interests.

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Tables
Table 1

Variable	WC	DA	SP	PD-CBT	PD-EXP	Test	Stat	p
N	187	95	38	25	29	-	-	-
Age in years (Mean [SD])	39.2 [11.4]	40.5 [10.4]	37.8 [13.2]	38.4 [11.9]	37.4 [11.9]	AN OV A	0.8 31	0.47 8
Gender (N male [%])	67 [35.8]	35 [36.8]	9 [23.7]	13 [52.0]	10 [34.5]	Chi squa re	5.3 5	0.14 8
Baseline CGI severity (Mean [SD])	4.70 [1.13]	4.83 [1.27]	4.16 [0.973]	4.80 [0.707]	4.86 [0.915]	AN OV A	3.8 0	0.01 12 *
Treatment duration in days (Mean [SD])	200 [184]	47.6 [32.8]	340 [152]	351 [118]	383 [137]	AN OV A	15 1	3.15 x10⁻⁴⁹ †
Follow-up duration in days (Mean [SD])	215 [62.7]	249 [72]	191 [41.4]	190 [35.4]	191 [44.7]	AN OV A	11. 4	4.32 x10⁻⁷ ‡
Psychoactive medication at baseline (N taking [%])	20 [10.7]	18 [18.9]	1 [2.63]	1 [4.00]	0 [0.00]	Fisher's exact test		0.00 247 §
Mental disorder comorbidities (N [%])	72 [38.5]	46 [48.4]	8 [21.1]	9 [36.0]	9 [31.0]	Chi squa re	9.5 8	0.02 25 ¶

Table 1: Demographic and treatment information on participants with genotype and/or expression data.

Post-hoc t-tests (variances assumed unequal; Bonferroni corrected significance threshold = 0.00834; significant results in bold):

* = SP lower: vs DA: $t=-3.29, p=0.00143$; vs PD-CBT: $t=-3.03, p=0.00361$; vs PD-EXP: $t=-3.04, p=0.00350$

† = DA shorter: vs SP: $t=-11.7, p=2.79 \times 10^{-14}$; vs PD-CBT: $t=-12.8, p=1.93 \times 10^{-12}$; vs PD-EXP: $t=-13.0, p=1.19 \times 10^{-13}$

‡ = DA longer: vs SP: $t=4.96, p=3.19 \times 10^{-6}$; vs PD-CBT: $t=4.86, p=6.93 \times 10^{-6}$; vs PD-EXP: $t=4.48, p=2.74 \times 10^{-5}$

§ = Higher rate in DA: vs SP: $t=3.38, p=9.47 \times 10^{-4}$; vs PD-CBT: $t=2.63, p=0.0103$; vs PD-EXP: $t=4.69, p=9.33 \times 10^{-6}$

¶ = Higher rate in DA: vs SP: $t=3.24, p=0.00174$; vs PD-CBT: $t=1.12, p=0.269$; vs PD-EXP: $t=1.71, p=0.0930$

Groups: WC = Whole cohort, DA = Dental anxiety, SP = specific phobia, PD-CBT = panic

disorder CBT,
PD-EXP = panic disorder exposure

Table 2

Correlations between clinical covariates and treatment response phenotypes for the whole cohort (N= 187)				
	Post-treatment		Follow-up	
Variable	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Age (years)	-0.0536	0.469	0.0212	0.816
Gender	0.045	0.543	0.0497	0.585
Baseline CGI severity	0.112	0.128	0.303	7x10⁻⁴
Treatment sessions	0.129	0.0818	0.106	0.255
Treatment duration (days)	0.117	0.111	0.0189	0.835
Follow-up duration (days)	-0.178	0.0409	-0.0411	0.6614
Psychoactive medication (use)	-0.205	0.00521	-0.203	0.0243
Other medication (use)	-0.0483	0.514	-0.117	0.200
Mental comorbidity (yes/no)	-0.210	0.00407	-0.188	0.0379
Body mass index	0.0572	0.440	0.0008	0.993
Smoker (yes/no)	-0.0317	0.687	0.0146	0.880

Table 2: Correlations between clinical covariates and treatment response phenotypes for the whole cohort (N= 187). Correlations with nominal significance ($p < 0.05$) are highlighted in bold.

Table 3

Response post treatment	Whole cohort	DA	SP	PD-CBT	PD-EXP	Test	Stat	<i>p</i>
N	185	95	37	24	29	-	-	-
% change in CGI (Mean [SD])	67.4 [34.6]	62.0 [32.1]	77 [39.5]	67.1 [37.1]	72.9 [31.8]	ANOVA	2.01	0.115
Improved (N [%])	165 [89.2]	84 [88.4]	33 [89.2]	21 [87.5]	27 [93.1]	Fisher's exact test		0.613
No change (N [%])	16 [8.65]	10 [10.5]	2 [5.41]	2 [8.33]	2 [6.90]			
Deteriorated (N [%])	4 [2.16]	1 [1.05]	2 [5.41]	1 [4.17]	0 [0.00]			
Response at follow-up	Whole cohort	DA	SP	PD-CBT	PD-EXP	Test	Stat	<i>P</i>
N	122	54	32	17	19	-	-	
% change in CGI (Mean [SD])	59.7 [46.2]	52.3 [54.5]	71.4 [37.9]	71.5 [30.7]	49.8 [41.2]	ANOVA	1.90	0.134
Improved (N [%])	101 [82.8]	42 [77.8]	28 [87.5]	16 [94.1]	15 [78.9]	Fisher's exact test		0.641
No change (N [%])	11 [9.02]	5 [9.26]	3 [9.38]	1 [5.88]	2 [10.5]			
Deteriorated (N [%])	10 [8.20]	7 [13.0]	1 [3.13]	0 [0.00]	2 [10.5]			

Table 3: Treatment response as percentage change in CGI-S, and grouped by improvement (percentage change positive), no change, and deterioration (percentage change negative), in the whole cohort and each treatment. Groups did not differ on treatment response by either measure.

Table 4

Associations between expression probes and treatment outcome					
Baseline – post-treatment					
Probe nuID	Gene	WGCNA module	Pearson's <i>r</i>	<i>p</i>	<i>q</i>
TkiT0uUa.K4LZ5M7h4	<i>FDFT1</i>	blue	0.282	2.34x10 ⁻⁴	0.756
0Z7unqF.KAuA5K4ggU	<i>FDFT1</i>	grey	0.241	0.00175	1
Eqx.SxEEVcI.VLrWJI	<i>IL18RAP</i>	grey	0.237	0.00211	1
Te4VV0giY1VcQvr17E	<i>RNASE6</i>	grey	-0.216	0.00515	1
QuynqD354KD6IAXvnk	<i>YIPF4</i>	grey	-0.214	0.00550	1
Baseline – follow-up					
Probe nuID	Gene	WGCNA module	Pearson's <i>r</i>	<i>p</i>	<i>q</i>
TXm4UjVovoAQ4ApVQo	<i>MYC</i>	grey	-0.346	2.17x10 ⁻⁴	0.702
Krrborr9LqDhB.rPoo	<i>HNRNPA1P33</i>	brown	-0.294	0.00180	1
Ew_iK7UunWqIb0nFeE	<i>AIF1</i>	grey	0.265	0.00518	1
6dFQSN.UitTroIYwV4	<i>MAL</i>	grey	-0.240	0.0115	1
T0upGOh1A5dC87MXtU	<i>PPP6C</i>	turquoise	0.235	0.0136	1

Table 4: Largest correlations between individual expression probes and the treatment response phenotypes. WGCNA modules refer to the data-driven clusters to which each probe belongs.

Table 5

Linkage-dependent and independent blood eQTLs		
FDR q threshold	# variants	# linkage-independent variants
0.01	26566	788
0.05	42868	1492
0.1	54795	2159
0.5	61799	2503

Table 5: Raw and clumped results from the expression QTL analysis, by false-discovery rate

Table 6

eQTL x treatment response predicting gene expression					
Baseline – post-treatment					
SNP	Probe nuID	Gene	Beta	<i>p</i>	<i>q</i>
rs11260538	94gYDdn0tHeWCmeGk0	<i>SDF4</i>	0.00394	7.86×10^{-4}	1
rs3129996	llGfH57t5ug93Xe1XU	<i>KIAA1949</i>	-0.00431	0.00126	1
rs16965033	onsnvop.hKDoejReHU	<i>HERPUD1</i>	-0.00528	0.00155	1
rs3743888	9Sft35eUe7g2mGiR5E	<i>AXIN1</i>	0.00270	0.00163	1
rs11850781	NoXN6F3SR7AMv_v_6Q	<i>NIN</i>	-0.00577	0.00164	1
Baseline – follow-up					
SNP	Probe nuID	Gene	Beta	<i>p</i>	<i>q</i>
rs10498246	fcV3S0U75If1e3op0U	<i>SP110</i>	-0.00414	2.23×10^{-5}	0.103
rs6701295	cXl3ddwDJC3qA16ri4	<i>SMG5</i>	0.00249	2.26×10^{-4}	0.523
rs1737046	Tt5huq2hqZcdZqzRSc	<i>HCG4</i>	-0.00318	3.99×10^{-4}	0.616
rs4602357	6oIooHit00T3Imfo5U	<i>CEP63</i>	0.00251	5.94×10^{-4}	0.688
rs12343854	fpmvXlHteCO4OrrGP0	<i>SEMA4D</i>	0.00231	9.24×10^{-4}	0.784

Table 6: Top 5 results from eQTL-outcome interactions predicting expression level.

No interactions are significant at $q < 0.05$.

Figure Legends

Figure 1: Correlations between expression profiles of module eigengenes from WGCNA and treatment phenotypes (and covariates). Positive correlations are shown in red, negative correlations in blue, with colour intensity indicating strength of correlation. No module expression profile is associated with a treatment response phenotype (all $p>0.05$).

